

Constitutively Active Receptors

File Name	Recentor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP I	3	, illinois and a second			7. 100
MSHR_mouse	melanocyte-stimulating hormone MSH	TMII	92 VSIVLETTIIL K	adenylyl cyclase activity/ HEK293, stably transfected	(Robbins, Nadeau et al. 1993)
CLASS A GROUP II				/ EDJanousgra	Bournele Gamble et al
5H1B_human	5-hydroxytryptamine _{1B}	C-terminus of IC3	313 RERKA <u>T</u> KTLGI K, R, Q	binding of [~3]417[3]7 CHQ-KI	(ratwels, Couple et al. 1999)
5H2A_human	5-hydroxytryptamine _{2A}	C-terminus of IC3	322 NEQKA <u>C</u> KVLGI K	IP production / COS-7	(Egan, Herrick-Davis et al. 1998)
2H2C_rat	5-hydroxytryptamine _{2C}	C-terminus of IC3	312 MEDIA 9 WATE OF	PI hydrolysis / COS-7	(Herrick-Davis, Egan et al. 1997)
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CT ASS A					
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A1AB_human	α _{1B} -adrenergic	TMDI	63 FAIVG <u>N</u> ILVIL	IP / COS-7	(Scheer, Fanelli et al. 1997)
	alpha 1B-AR		ď		
	-	junction between TMDIII and IC2	142 CAISI <u>D</u> RYIGV A		
A1AB_human	α _{1B} -adrenergic	junction between TMDIII and IC2	143 CAISID <u>R</u> YIGV K	IP / COS-7	(Scheer, Costa et al. 2000)
A1AB_human	alpna 15-Ar. α ₁₈ -adrenergic	TMIII	128 AVDVL <u>C</u> CTASI F	IP/COS-1	(Perez, Hwa et al. 1996)
		carboxyl end of IC3	293 REKKA <u>A</u> KTLGI	IP arachidonic acid release	
		TMV	E 204	IP / COS-1	(Hwa, Gaivin et al. 1997)
		and the second	Λ		
A1AB_human	α _{1B} -adrenergic	C-terminal IC3	SREKKAAKT X=19 different substitutions	PI / COS-7	(Kjelsberg, Cotecchia et al. 1992)
A1AB_human	α _{1B} -adrenergic	C-terminus IC3	288 293 KFSREKAAKTLGI KHI	PI hydrolysis / rat fibroblast	(Allen, Lefkowitz et al. 1991)
A2AA_human	α ₂ C10-adrenergic	C-terminal IC3 loop	TO TO	adenylyl cyclase inhibition / HEK293	(Ren, Kurose et al. 1993)
ACM1_human	alpha-2AAR muscarinic Hm1	C-terminal IC3 loop junction	360 SLVKEKKAARTLS	PI / HEK(U293)	(Högger, Shockley et al. 1995)
ACM2-human	muscarinic acetylcholine M1 muscarinic acetylcholine M2	junction of IC3 and TMVI	XKVTRTIL†A	IP production, inhibition of cAMP production /	(Liu, Blin et al. 1996)

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CLASS A					
ACM3_rat	m3 muscarinic (rat)	TMVI	507 TWTPY <u>N</u> IMVLVNT S	IP/COS-7	(Blüml, Mutschler et al. 1994)
ACM5_human	mS muscarinic acetylcholine M5	N-terminus to TMII TMVI	chimera composed of m2 1-69 m5 77-445 m2 301-466	β-gal / NIH 3T3	(Burstein, Spalding et al. 1996)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	TMVI	A59 465 AILLA EIITW TPYNI MVLVST M L H C V S F	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1998)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	junction of TMVI and EC3	465 YNIMVLV <u>S</u> TFCDKCV X=V,F,R,K,+more	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1997)
BIAR_human	β ₁ -adrenergic	C-terminus	389 RKAFQGLLCCA R	adenylyl cyclase; agonist binding / CHW	(Mason, Moore et al. 1999)
B2AR_human	β ₂ -adrenergic beta-2AR	C-terminal IC3 loop	266 272 FC <u>LK</u> BHKALKTLGI SR K A	adenylyl cyclase activation; agonist binding affinity / COS-7 or CHO	(Samama, Cotecchia et al. 1993); (Lefkowitz, Cotecchia et al. 1993)
DADR_human	dopamine D1A	carboxyl terminal IC3	264 SFKMS <u>FKR</u> ETKVLKT I K 288 from D1B receptor APDTSIKKETKVLKT	adenylyl cyclase; cAMP accumulation / HEK293	(Charpentier, Jarvie et al. 1996)
DADR_human	dopamine D1	TMVI	286 FVCCW <u>L</u> PFFIL A	CAMP accumulation / COS-7	(Cho, Taylor et al. 1996)
HH2R_rat	histamine H ₂	IC2	115 FMISL <u>D</u> RYCAV N,A	cAMP production / HEK-293	(Alewijnse, Timmerman et al. 2000)
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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Keierence
CLASS A GROUP III		is to			
OPSD_human	opsin	TMII	90 FMVLGGFTSTLY	transducin; phosphorylation by	(Rim and Oprian 1995)
	rhodopsin	A34, 444	0	rhodopsin kinase / COS	
		IMITI	GCNLEGFFAT		
			0		
•		TMVII	292 296 WHITE REPARENTY		
_			MILPAFFASSAMI E G,E,M		
			²⁹² Ala neutral a.a converted to		
			carboxylate and competes with ¹¹³ Glu for salt bridge with ²⁹⁶ Lys		
OPSD human	Opsin	TMIII	134	transducin; radioligand	(Acharya and Karnik
1			VVLAIERYVVV	binding / COS	1996)
	rhodopsin		I,Q,S		
OPSD human	opsin	TM6	257	transducin, GTPyS	(Han, Smith et al. 1998)
l	4		RMVIIMVIAFL	uptake / COS	
	rhodopsin		Y,N		
		plus TM3	plus G113Q		
OPSD_human	nisqo	TMVII	296	transducin; radioligand	(Govardhan and Oprian
			FAFFAGA	COO / Stringing	(Cohen. Yang et al. 1993)
-1	rhodopsın		X=E,M natural mutants		
_			+ 10 different a.a. substitutions		
			disrupts critical salt bridge between ²⁹⁶ Lys(TMVII) and ¹¹³ Glu(TMIII)		
		3	134		
		104	VVLAIERYVVV Q		(Cohen, Yang et al. 1993)
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erence	كالمعاد الإسارة والمائد		(Marie Koch et al. 1999)		ŧ						
			Γ	IP production / CCS-/							
	Sequence			113	ISSI	A	256 256	LLFIICMLFFQI	ž.		_
	Mutation Site			2227 100		TMVI					
	Descritor	Kecepion			bradykinin B ₂	B2 bradvkinin	DZ Utacykamia	DN-Z			
	M. W.	File Name	CLASS A	GROUP IV	BRB2_human						

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				Aesay / Cells	Reference
File Name	Receptor	Mutation Site	Sednence	1	
CLASSA	المعالف المدار الذي المراز				
GROUP V AG2R_rat	ATıA	TMIII	111 ASVSFMLYASV	phospholipase C; IP production / COS-7	(Groblewski, Maigref et al. 1997)
	Type-1A angiotensis II		disrupts 111Asn(TMIII) -		4
AG2R_rat	ATıA	C-terminus of TM7	i	IP production / HEK- 293; intrcellular Ca ²⁺	(Parnot, Bardin et al. 2000)
	Type-1A angiotensis II	other multiple mutations	O	Di production	(Amatruda, Dragas-
FMLR_human	formylmethionylleucylphenylal anine (fMLPR)	ICI	JI LV <u>I</u> WV <u>AGFRMTHTVTTISY</u> LNKAVA LVVWVTAFEAKRTINAIWFLNLAVA	phospholipase C stimulation / COS-7	Graonic et al. 1995)
			(K above conflicts with SWISS-PROT database)		-
IL8B_human	interleukin-8 receptor B	IC2	138 ACISV <u>D</u> RYLAIVH	IP production; Carmoblization and actin	(Burger, Burger et al. 1999)
	CXCR-2 chemokine			polymerization / NIH 3T3	
LSHR_human	luteinizing hormone (LH)	IC3	564 MATNK <u>D</u> TKIAKK G	cAMP production / HEK293	(Kụdo, Osuga et al. 1996)
LSHR_human	luteinizing hormone (LH)	TMVI	578 ILIFIDFTCMA G	cAMP production / COS-7	(Shenker, Laue et al. 1993)
LSHR_human	luteinizing hormone (LH)	TM6	571 577 KIAKK <u>W</u> AILIF <u>T</u> DFTCM I I	cAMP production / COS-7	(Kosugi, Van Dop et al. 1995)
LSHR_rat	luteinizing hormone / human chorionic gonadotropin	TMVI	556 ILIFTDFTCMA G, Y	cAMP production / HEK 293T	(Bradbury, Kawate et al. 1997; Bradbury and Menon 1999)
OPRD_mouse	delta opiod receptor	TM3	128 KVLSI <u>D</u> YYNMF A, K, H	adenylyl cyclase inhibition / COS-7	(Cavalli, Babey et al. 1999) (Eggell: Bothier et al.
OXYR_human	oxytocin	ICZ	137 LMSLDRCLAIC A	Ir production / COS-7	(Faircin, 2aroix et a 1999)

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PAFR_human	platelet-activating factor (PAF)	C-terminus of IC3	231	IP production / COS-7	(Parent, Le Gouill et al.
-		-	EVAKKALMINOLI VIIAV R	_	
PAFR_human	platelet-activating factor (PAF)	TMIII	100 CLFFINTYCSV A	arachnidonate release, IP production, adenylyl cylcase inhibition / CHO	(Ishii, Izumi et al. 1997)
PE23_human	prostaglandin E ₃ , EP3III EP3IV	C-terminal tail	360 FCOBEFWGN FCOMRKRILREOBEFWGN Truncated	inhibition of adenylyl cyclase / CHO-K1	(Jin, Mao et al. 1997)
PE23_mouse	prostaglandin E ₃ EP3	carboxyl-terminal tail	336 KILLRKFCQ <u>IRDHT</u> (3α) <u>MMNHL</u> (3β) ^truncated	inhibition of adenylate cyclase / CHQ, stably expressed	(Hasegawa, Negishi et al. 1996)
THRR_human	thrombin	BC2 loop	259 268 CHDVLNETLLEGYYAYY DLKD KDF I	45Ca 2+ efflux, PI hydrolysis, reporter gene induction / COS-7	(Nanevicz, Wang et al. 1996)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	BC1	486 YYNHA <u>I</u> DWQTG F,M	inositol phosphate diacylglycerol cascade / COS-7	(Parma, Van Sande et al. 1995)
		BC2	568 YAKVS <u>I</u> CLPMD T		
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMIII	509 ASELS <u>V</u> YTLTV A	adenylyl cyclase activation / COS-7	(Duprez, Parma et al. 1994)
		TMVII	672 YPLNS <u>C</u> ANPFL Y		•
TSHR_human	thyrotropin (TSHR)	TMV	597 VAFVI <u>V</u> CCHV L	cAMP formation / CQS-7 cells	(Esapa, Duprez et al. 1999)
TSHR_hyman	thyrotropin (TSHR)	TMVII	677 CANPFLYAIFT V	cAMP formation / CHO cells	(Russo, Wong et al. 1999)
TSHR_human	thyroid stimulating hormone thyroid stimulating hormone	IC3	613 621 VRNPQ <u>YNPGDKDTK</u> IAK deletion	cAMP formation / COS-7	(Wonerow, Schoneberg et al. 1998)

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TSHR_human th	thyrotropin (TSHR)	IC3 / TMVI	623 632 KDTKIAKRMAVLIFIDFICM	cAMP activation / COS-7	(Paschke, Tonacchera et al. 1994)
V2R_human	thyroid stimulating hormone vasopressin V2	IC2	136 LAMTL <u>D</u> RHRAI	cAMP formation / CQS-7	(Morin, Cotte et al. 1998)
			А	•	

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		-7300	Commondo	Assav / Cells	Reference
File Name	Receptor	Mutation Site			المنظف معافدين والإنتاج في الإنتاج المناطقة
CLASS B					(1004) 1 The same of the
CALR_human	hyman calcitonin hCTR-1	wild type (native) protein		adenylyl cyclase cAMP production / COS-1	(Conen, 1 naw et al. 1991)
CLASS B					
PTRR_human	parathyroid hormone PTH / PTH-related peptide	junction of IC1 and TMII	223 TRNYI <u>H</u> MHLFL R, K	cAMP accumulation / COS-7	(Schipani, Jensen et al. 1997)
		junction of IC3 and TMVI	410 KLIKS <u>T</u> LVLMP C, others		
CLASS B					
GROUP III GIPR_human	glucose-dependent insulinotropic peptide (GIP-R)	TMVI	340 VFAPV <u>T</u> EBQAR P	cAMP production / L293	(Tseng and Lin 1997)
GLR_rat	glucagon	junction of IC loop1 and TMII	178 TRNYI <u>H</u> GNLFA R	cAMP accumulation / COS-7	(Hjorth, Orskov et al. 1998)
		IC end of TMVI	352 RLARS <u>T</u> LTLIP A		
VIPR_human	vasoactive intestinal peptide 1 (VIP)	junction of IC loop 1 and TMII	178 RNYIHMHLFI R requires functional integrity of the N-terminal EC domain	cAMP production / COS-7 or CHO	(Gaudin, Maoret et al. 1998) (Gaudin, Rouyer-Fessard et al. 1998)
		junction of IC loop 3 and TMVI	343 LARS <u>T</u> LLLIP X= K, P		

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Reference	, 15	VI Onelding of al	(Jensen, Spaiding et ai.	2000)						*			•	
A seast / Colle	ASSAY / COLO		IP / tsA											
	Sequence			in'	multiple combinations			X ¹					/	
	Mutation Site		701	N-terminal CC										
	Recentor			calcium-sensing]	
	File Name	T. II. L'ANTING	CLASSC	CASR_human										

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File Name	Receptor	Mutation Site	Sed hence		
CLASS D		2.		1 - 4 Joseph Company	(Olesnicky Brown et al.
674283	pheromone	TM6	229 DI.SAVOTVI.GT	neterotogous yeast assay	1999)
RCB2			C.		
C. cinereus		27/4	258	lacZ reporter gene	(Konopka, Margarit et al.
STE2_yeast	pheromone α-factor	TWO	QSLLVPSIIFI		(9661
			LL		3
CTTES	aboromone & factor	double mutations TM5	223	lacZ reporter gene /	(Dube, DeCostanzo et al.
157-36436	pieroinque Cracco		MSFVLVVK	yeast	2000)
		and	ย		
_			247 251	****	
		TM6	DSFHILLIMECOSLL		
			22 22		
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			double mutations		
				O antimotocidada	(Roone, Davis et al. 1993)
STE3 yeast	pheromone a-factor	_IC3	144	p-garaciosidase	
)			DVRDILHCINS		
			252 250	R-coloctosidase	(Sommers, Martin et al.
STE2 yeast	pheromone α-factor	l IM6		Paracionata	2000)
			LANGUSALIVESTIFF		
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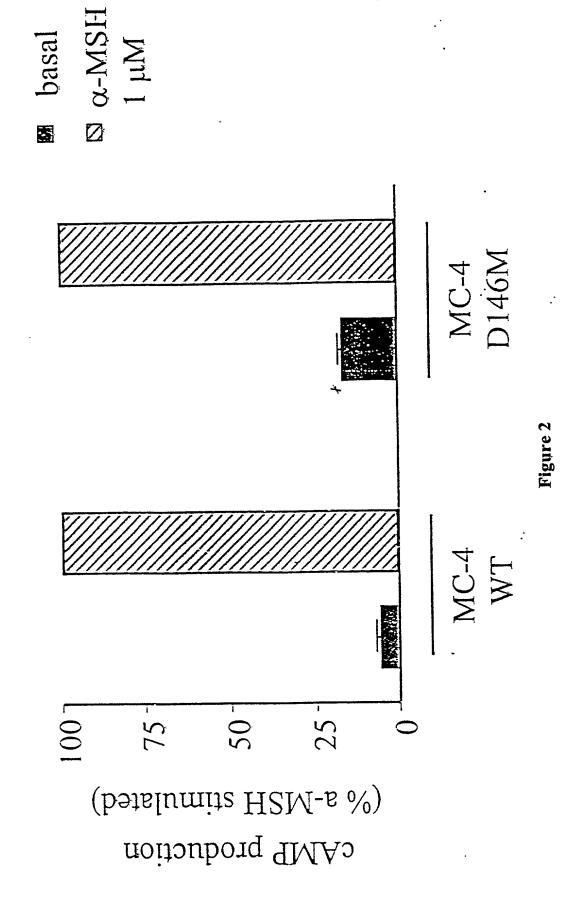
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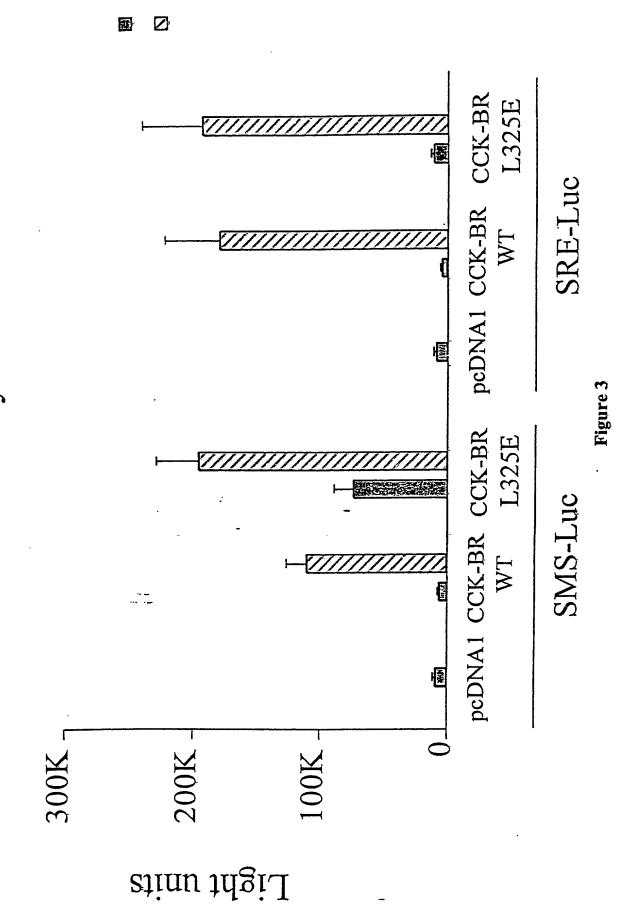
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A Point Mutation Enhances MC-4 Receptor Constitutive Activity



Light Emission Induced by the WT CCK-BR vs. a Constitutively Active Mutant



CCK-8

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A Point Mutation Confers Constitutive Activity to the Rat µ Opiod Receptor

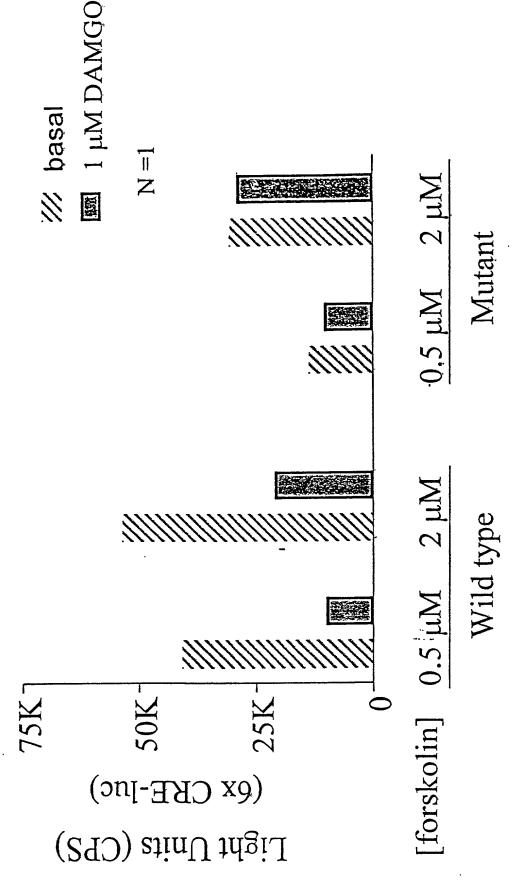


Figure 4

Forskolin Stimulated HEK293 Cells Transfected With pcDNA1 and a CRE-luc Construct

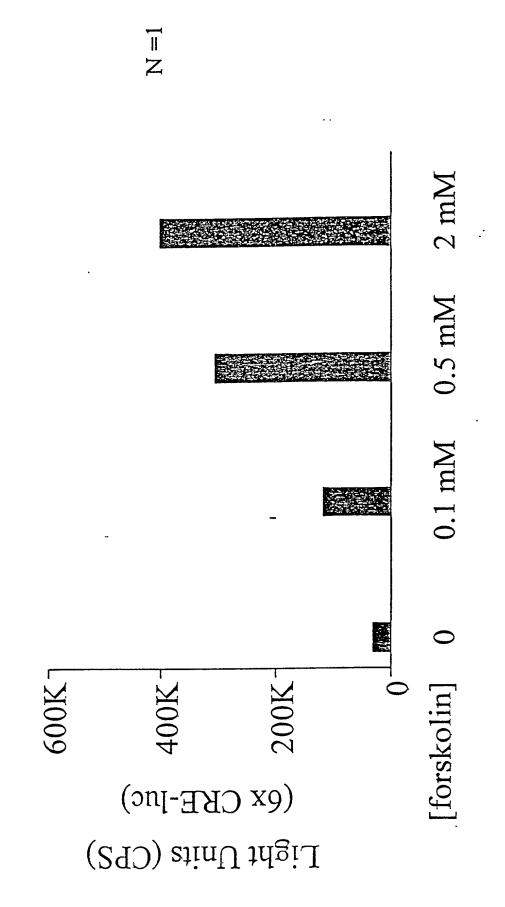


Figure 5

The Rat μ Opioid Receptor Signals Through Gai

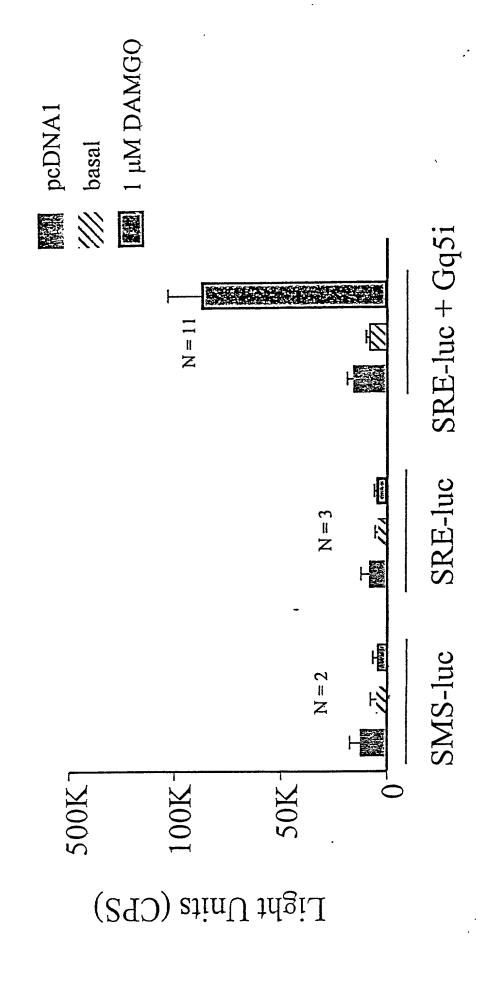
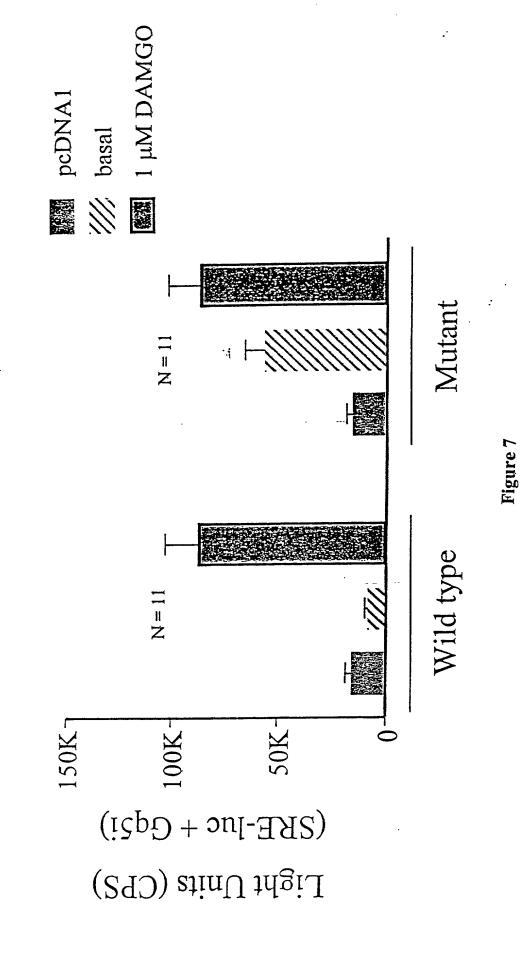


Figure 6

A Point Mutation Confers Constitutive Activity to the Rat µ Opioid Receptor



Target Residues Within Class I GPCRs

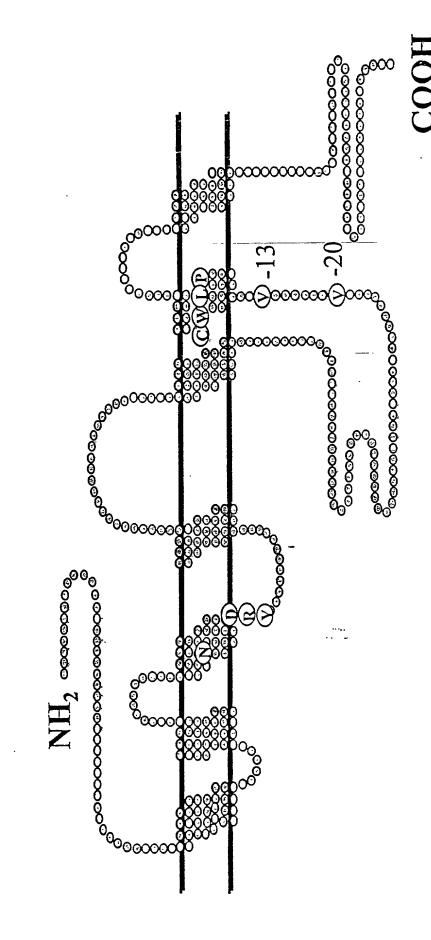
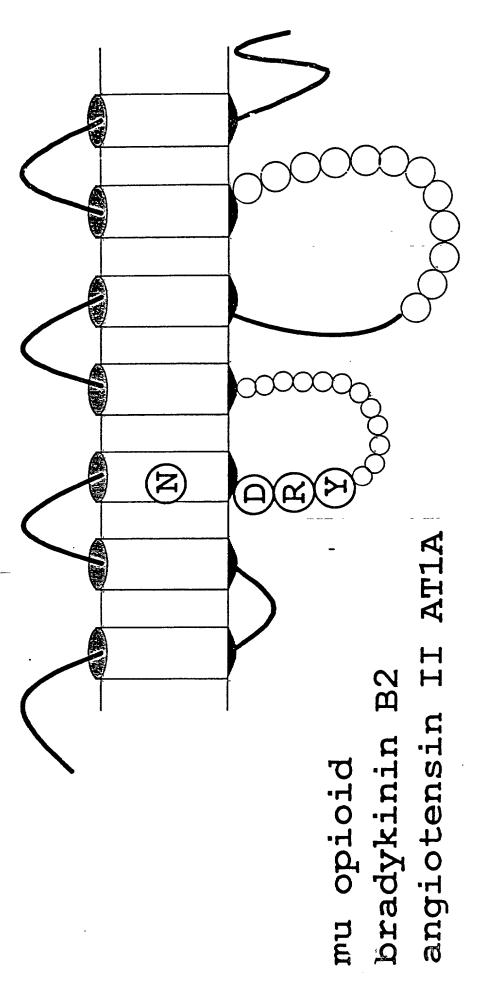


Figure 8

for Mutation Induced Constitutive Activity TMD III Asn (-14 from DRY) is a Target



The 'DRY' Motif is a Target for Mutation Induced Constitutive Activity

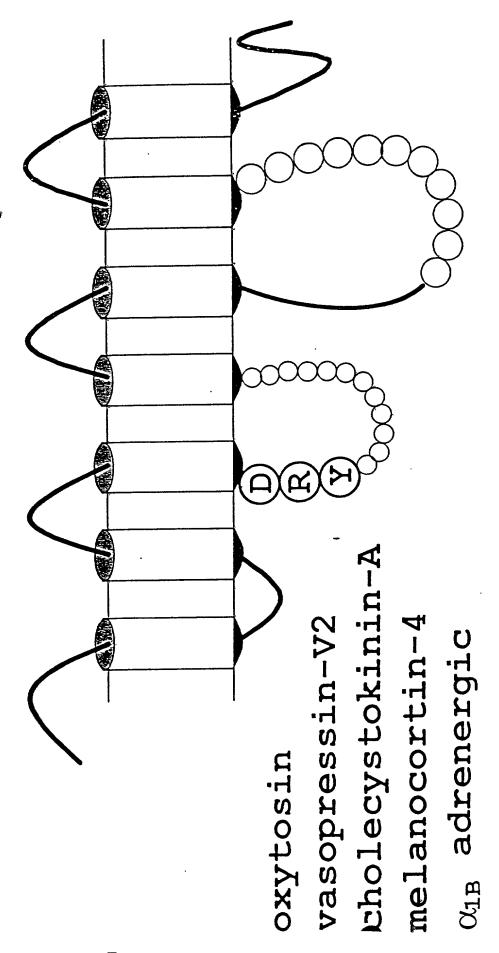
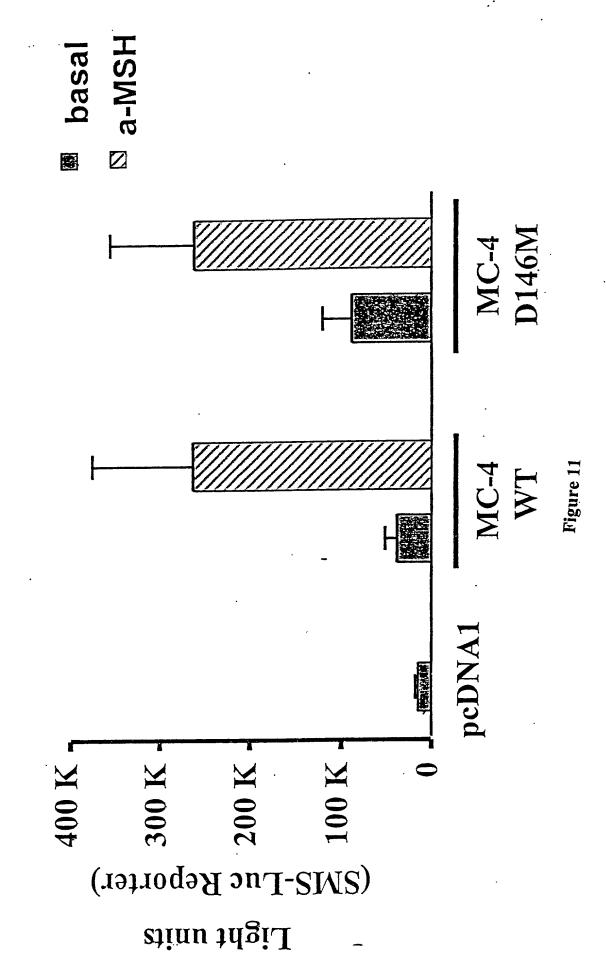


Figure 10

A Point Mutation Enhances MC-4 Receptor Constitutive Activity



The -13 Position is a Target for Mutation Induced Constitutive Activity

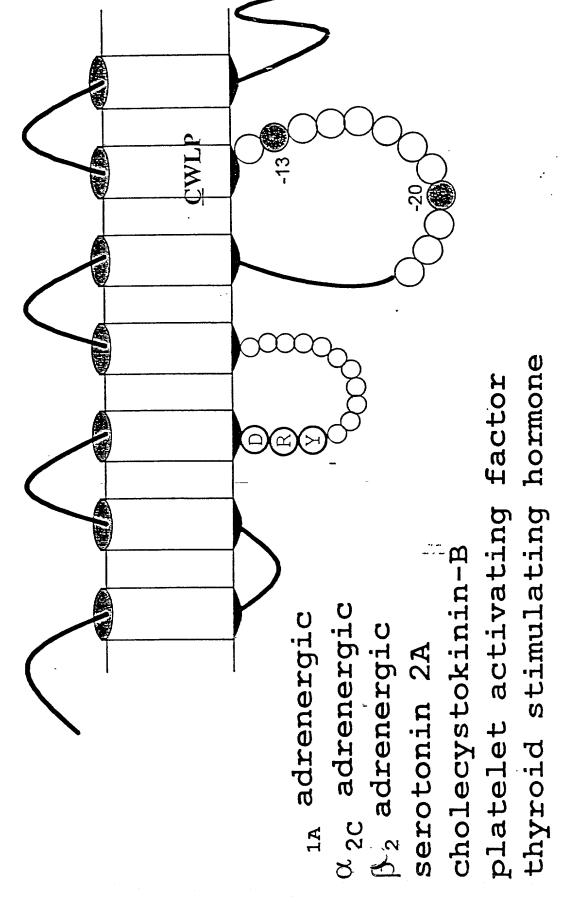


Figure 1.

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ATIA 1 -----MALNSSAEDEIKRI
     BK-2
                                1 -----MFSPWKISMFLSVREDSVPTTASFSWMLNVTLOGPTLNG.TFA
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orkr 49 Lepanistan...pysitanysyvervenkensikynevinkyrkykvarnivenladada
orm 59 Cpptgs...smitantimanysinycyveneensikynykvinkyrkykrarnivenhalada
ormr 57 Cpqtgs...smytantimanysinycyveneensikynyvyvinkyrkykrarnivenhalada
ord 37 ppgaksassialadautanysaycaveneensikyvinyrykykrarnivenhalada
atia 16 DDCpkagrhsyifyvyptiysinsiyveneensikyviviyyfymkeikyvasyelinladadi
bk-2 45 skcpqvewlgwlntiqppflwviryelathenifyrsyfclhksscivaeivignilaaadi
  ork 107 IVIIIIMPROSTVYIMI SWPEGDILCKOVISIDYYMVETSIET TYMSVDRYIAVCHPVK
orkr 107 IVIIIIMPROSAVIIMI SWPEGDILCKOVISIDYYMVETSIET TYMSVDRYIAVCHPVK
ormr 118 IAISTIPPOSAVIIMI MWPEGTILCKOVISIDYYMVETSIET CTWSVDRYIAVCHPVK
ormr 116 IAISTIPPOSAKVIME MWPEGTILCKOVISIDYYMVETSIET CTWSVDRYIAVCHPVK
ord 97 IAISTIPPOSAKVIME MWPEGNILCKOVISIDYYMVETSIET TYMSVDRYIAVCHPVK
AT1a 76 CFLLTDLWAVYTAMEYRWPEGNILCKOVASASVTENTVASJELITCISEDRYMATIVHPMK
IK-2 105 ILACGIAPIWATISNNFDWLBGETLCKOVASISVTENTVASJELITCISEDRYMATIVHPMK
                                                                                                                                                                            -14 from DRY
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67 kr 166 ALDSZIPLKAKO NIE WOLSSSYGDSANYLGGIKVR. BDVDWIEGSLOFPDDEVSWYD

67 mr 177 ALDSZIPRNAKO NIE NIE SANGERWYF VATUKYR. O. GSID GWITTESHPTW. WWD

67 d 156 ALDSZIPRNAKO NIE WALASGEGET WWAVIZPR. D. GAVVOM OFFSPSW. WWD

AT1a 136 SRLRZYMLVAKOTO II WWAGLASD PAWDHRNV. YFIBNTNUTVCAFHYESRN. STLP

BK-2 165 MGRMRGVRWAKO YSTVIWGC ILLISSPYTV FRIMKEYSDEGHNVTACVI SVPS. . LIWE
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orm 232 NLFK CVF FARMIPVA ITVCVGMILRAKSVRILSGSKEKORNLERITRIVLVVVAVF
orm 230 NLFK CVF FARMIPVA ITVCVGMILRAKSVRILSGSKEKORNLERITRIVLVVVAVF
ord 211 TVTKICVF FARMIPIA ITVCVGMILRAKSVRILSGSKEKORNLERITRIVLVVVAVF
AT1a 193 IGIGETKNILGS FFFF IN ITSVIJWKALKKAYE I OKNKPRNDD ... IFRE I MAIJVLEF
BK-2 222 VFTNVLINVVGRADD. I SVITTCINO I MOVLENNEMOKFKE I OTE . RRATVI VLVVIČLIGE
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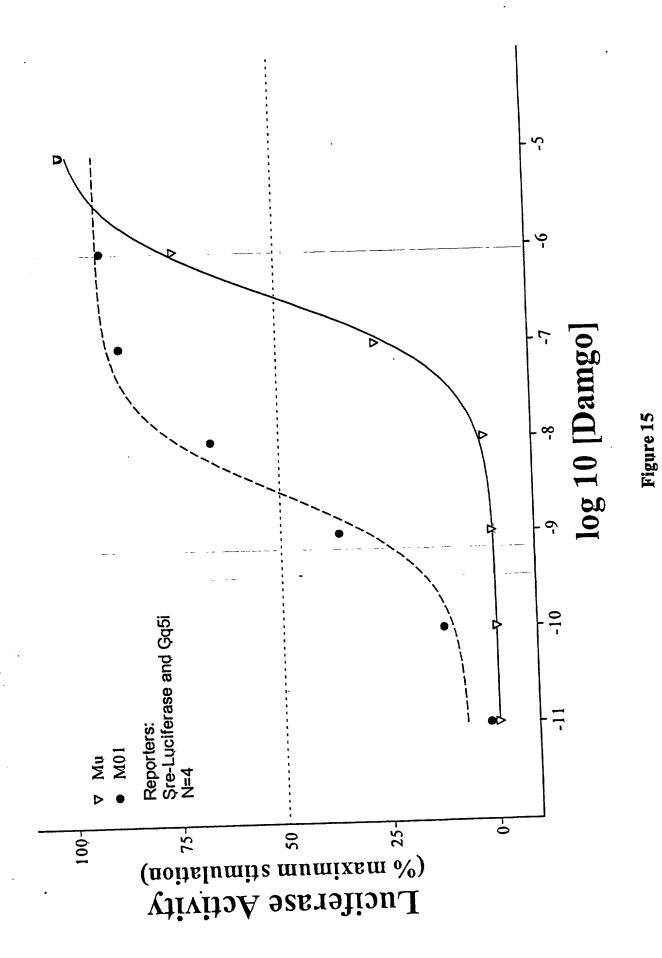
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ATLA 250 FFS MVBHOLETF MOVING UGVIHDCKISDIVDTAMPITIC TAYFUNG LNPLYVAELGKEF

EK-2 280 THICKLEFOUSTF MOTHER GILSSCODER INDVITQUAS FVEY SNS CLAPALY VIVGKRE
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mORmouse 1 MDSSAGEGNISDCSDPIA.PASCSPA.ECSNUMLSHVDGNOSDFCGPNRYGLGGSHSLC
mORrat 1 MDSSTGEGNISDCSDPIA.QASCSPA.ECSNUMLSHVDGNOSDFCGLNRYGLGGNDSIC
mORbovin 1 MDSGAVETNASNCUDFFTHPSSCSPAPSESSNUMFSHIJGNLSDFCGPNRYELGGSDRIC
mORhuman 1 MDSSADERWASNCUDAIAY.SSCSPAFSESSNUMFSHIJGNLSDFCGPNRYDLGGRDSIC
mORPig 1 MDSSADERWASNCUDFFSPSSMCSPVPSESSNUMFSHIJGNLSDFCGPNRYDLGGRDSIC
mORWS 1 MESS..GNISDFLYPIS...NEVMS...NSSVLCRNFSNSTSFLNMNGSSRDSTD
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           ATla
                                                                             1 -----mfsewkismflevredsvpttasfsadmlnvtlogetlng.tfacskc
           RK-2
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        morrat 58 porspswyrattivalysivcvvglegmelwwyvivrytkwkratwiyifwlaladala
morbovin 61 psacspswyrattiwalysivcvvglegmelwwyvivrytkwkratwiyifwlaladala
morhuman 60 pprospswyrattiwalysivcvvglegmelwwyvivrytkwkratwiyifwlaladala
        morpig 61 EPIESPSWITATIWALVSIVOVGASGUSVMVVIVRVIKMKIATNIVIENLALADALA
morws 48 EODKAB, WITATITTIVSIVOVGASGUSVMVVIGRYTKMKVATNIVIENLALADALA
ATIA 19 EKAGRESYIEVM. IPITAYSITEVVGASGUSLAVATVIYIYMKAKVASVE LALALADIGE
EK-2 48 EOVEWLGWINTI. OPPFLWVIEVEATLENIEVISVFCLHKSSOTVAETVIGNIAAADLIL
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EK-2 107 ACGLPFWATTISNNFDWLFGETLCRWVNFTISMNIVSSICFLWLWSCDRYLALVKTWSMG
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      RORMOUSE 230 NILKICYFIFAFIMPVLITTVCXGLMILRLKSVRWLSGSKEKDRNLRRITRMVLVVVAVF
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  mORpig 293 TVCMTPILLV KALT PRITTORYSMHECIALGYINSCANEVAYAFADENE MORWS 286 TECHTPILLV KALT PRITTORYSMHECIALGYINSCANEVAYAFADENE AT1a 250 FFSWYEHOUSTFIDVLO GVIHDCKISDIVDTAMPITIOTYFWACANETAYAYAFADENE EK-2 280 THEWLEFOUSTFIDTHRUGILSSCODERIIDVITQIASFWHYENSCANETAYIVIVGKRE
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Figure 14



An Intracellular Point Mutation Results in Loss of Ligand-Induced Function

IP Production / ³H Inositol incorporated

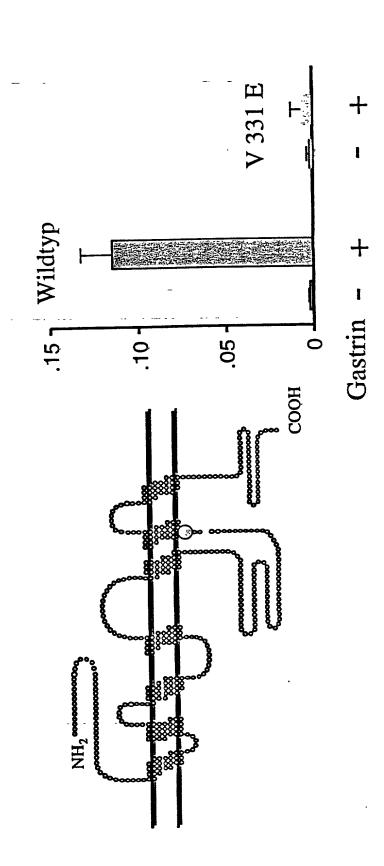


Figure 16

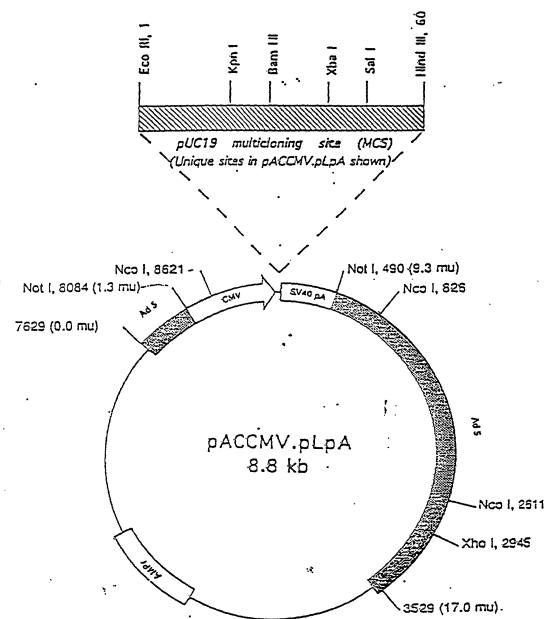


Figure 17